Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the investigation of **Furcelleran** in the developing chicken embryo 2/28/74

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carrageenan

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of FURCELLERAN in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken Embryo:

FURCELLERAN

PROTOCOL:

Furcelleran (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the albumen at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3, 4). The route of albumen, instead of the usual air cell, was chosen because of the fact that the administered furcelleran solution formed globular coagulates as soon as it was injected into the air cell, and absorption through the embryonic membrane was not conceivable.

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group. All abnormalities were tabulated.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (5). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Furcelleran was found to be quite embryotoxic when administered to the embryos under all conditions of the test. The toxicity was significantly (P=0.05) greater than in the solvent-treated eggs at all dose levels tested without exception. Probit analysis resulted in an LD_{50} of 1.611 mg/egg at 0 hours (Table 1) and that of 1.449 mg/egg at 96 hours (Table 2) via the albumen. Yolk treatment at 0 hours resulted in an LC_{50} of 1.089 mg/egg (Table 3), while the same treatment at 96 hours resulted in a line whose slope was not significantly different from zero (Table 4).

Abnormal birds were seen under all conditions of the test, but the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from the solvent background (P=0.05). Of the 217 untreated control embryos, only one was abnormal with curved toes.

ALBUMEN AT 0 HOURS: At 5.0 mg/egg there were three abnormal birds with either celosomia, abnormal maxillary curvature, or curved toes. At 1.0 mg/egg a total of six birds exhibited abnormalities; one bird each with celosomia, exophthalmic eyes, agenesis of the foot, abnormal

maxillary curvature, and two birds with hip contracture. At 0.5 mg/egg only one bird was abnormal with celosomia. At 0.25 mg/egg two birds showed either celosomia or curved toes. At 0.1 mg one bird had curved toes. The solvent-treated group had three abnormal birds, all showing curved toes.

ALBUMEN AT 96 HOURS: At 5.0 mg/egg two birds displayed either hip contracture or celosomia. At 1.0 mg/egg one bird had hip contracture and another bird showed curved toes. At 0.25 mg/egg both of the two abnormal birds had hip contracture. The solvent-treated group had one bird with curved toes.

YOLK AT 0 HOURS: At 5.0 mg/egg only one bird showed an abnormality, exophthalmic eyes. At 1.0 mg/egg there was a total of three abnormal birds with either abnormal maxillary curvature, hip contracture or curved toes. At. 0.5 mg one bird had curved toes and two other birds showed celosomia. At 0.25 mg/egg two birds had hip contracture. The solvent-treated group had two birds with curved toes.

YOLK AT 96 HOURS: At 5.0 mg/egg three birds were abnormal each with either hip contracture, celosomia or an abnormally curved maxilla. At 1.0 mg/egg one bird had hip contracture and the other had celosomia. Only one bird was abnormal with hip contracture at 0.5 mg/egg. At 0.25 mg/egg, one bird had celosomia, the other had supernumerary limbs and the third bird had curled toes. The solvent-treated group had four birds with curled toes and one bird had hip contracture.

Histological examinations of the major organs revealed no evidence of consistent change due to either the dose level of the substance administered or the mode of the treatment.

From these results it cannot be concluded that furcelleran is teratogenic to the chicken embryo. However, it is a noteworthy finding that in both the albumen and yolk sac administration routes, with the higher dose levels (5.0 mg and 1.0 mg/egg) at 0 hours there were anomalies of the eye and maxilla which were not observed in the solvent-treated embryos. This may warrant an additional study of the effect of furcelleran on embryonic development.

1. Furcelleran (Edible), 44 C, FDA Order 376-73 (F), The Burton Co., Nutley, N.J. 07110

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Hanan, E.B. Am. J. Anat. 38:423-450, 1927

5. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Furcelleran

Albumen at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
5.0	100	122	73.77*	2.45	2.45
1.0	20	125	60.00*	4.80	4.80
0.5	10	120	45.83*	1.66	0.83
0.25	5	126	30.15*	2.38	2.38
0.1	2	64	35.93*	1.56	1.56
Water		110	11.81	2.72	2.72
Control		217	9.67	0.46	0.46

LC_{30} 0.531 mg/egg (10.630 mg/kg)

LC_{50} 1.611 mg/egg (32.235 mg/kg)

LC_{90} 24.252 mg/egg (485.059 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Furcelleran

Albumen at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
5.0	100	100	69.00*	3.00	2.00
1.0	20	116	58.62*	1.72	1.72
0.5	10	97	53.60*	0	0
0.25	5	106	27.35*	2.83	1.88
0.1	2	54	40.74*	0	0
Water		151	15.23	0.66	0.66
Control		217	9.67	0.46	0.46

LC_{30} 0.362 mg/egg (7.244 mg/kg)

LC_{50} 1.449 mg/egg (28.986 mg/kg)

LC_{90} 42.937 mg/egg (858.755 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 3

Furcelleran

Yolk at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
5.0	100	64	78.12*	3.12	1.56
1.0	20	64	65.62*	4.68	4.68
0.5	10	64	45.31*	4.68	4.68
0.25	5	64	37.50*	3.12	3.12
Water		131	19.08	1.52	1.52
Control		217	9.67	0.46	0.46

LC_{30} 0.345 mg/egg (6.906 mg/kg)

LC_{50} 1.089 mg/egg (21.784 mg/kg)

LC_{90} 18.044 mg/egg (360.890 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 4

Furcelleran

Yolk at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
5.0	100	58	67.24*	5.17	5.17
1.0	20	58	56.89*	5.17	3.44
0.5	10	58	34.48*	1.72	1.72
0.25	5	58	31.03*	5.17	5.17
Water		129	17.05	3.87	3.87
Control		217	9.67	0.46	0.46

F Calculated $<$ F (0.05)

*Significantly different from solvent $P \leq 0.05$